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Evaluation of water quality during successive severe drought years within *Microcystis* blooms using fish embryo toxicity tests for the San Francisco Estuary, California

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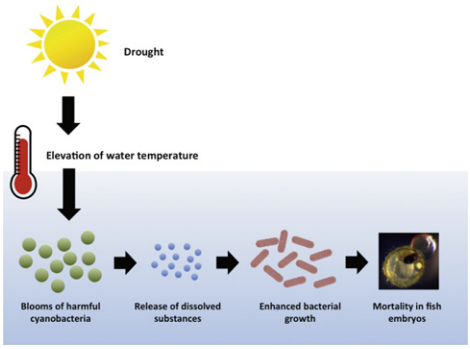
HIGHLIGHTS

- California experienced a severe multiple year drought in 2014 and 2015.
- Water quality was tested by fish embryo toxicity tests using Delta Smelt and Medaka.
- High mortality was observed in Medaka embryo in conjunction with the growth of *Aeromonas*.
- Growth of *Aeromonas* was enhanced by *Microcystis* cell lysate.
- Cyanobacterial blooms may be a cause of water quality deterioration by enhancing bacterial growth in the wild.

GRAPHICAL ABSTRACT



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ABSTRACT

In the San Francisco Estuary, California, the largest estuary on the Pacific Coast of North America, the frequency and intensity of drought and associated cyanobacteria blooms are predicted to increase with climate change. To assess the impact of water quality conditions on estuarine fish health during successive severe drought years with *Microcystis* blooms, we performed fish embryo toxicity testing with Delta Smelt and Medaka. Fish embryos were exposed to filtered ambient water collected from the San Francisco Estuary during the *Microcystis* bloom season in 2014 and 2015, the third and fourth most severe recorded drought years in California. Medaka embryos incubated in filtered ambient waters exhibited high mortality rates (>77%), which was mainly due to bacterial growth. Medaka mortality data was negatively correlated with chloride, and positively correlated with water temperature, total and dissolved organic carbon, and ambient and net chlorophyll *a* concentration. Delta Smelt embryo mortality rates were lower (<42%) and no prominent seasonal or geographic trend was observed. There was no significant correlation between the Delta Smelt mortality data and water quality parameters. *Aeromonas* was the dominant bacteria that adversely affected Medaka. The growth of *Aeromonas* was suppressed when salinity was greater than or equal to 1 psu and resulted in a significant reduction in mortality rate. Bacterial growth test demonstrated that the lysate of *Microcystis* cells enhanced the growth of *Aeromonas*. Toxin production by *Microcystis* is a major environmental concern, however, we conclude that dissolved substances released from *Microcystis* blooms could result in water quality deterioration by promoting growth of bacteria. Furthermore, a distinctive developmental deformity was observed in Medaka during the toxicity tests;

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somite formation was inhibited at the same time that cardiogenesis occurred and the functional heart was observed to be beating. The exact cause of the embryonic developmental deformity is still unknown.

1. Introduction

For the San Francisco Estuary (SFE) in the state of California, the largest estuary along the Pacific Coast of North America, climate change was associated with a severe multiple year drought that began in 2012 and persisted through 2015 (Cayan et al., 2009). The 2012 to 2015 drought in California was characterized by below average precipitation and above average atmospheric temperature (USEPA, 2015). These atmospheric conditions also produced a decrease in freshwater inflow and increase in water temperature, which favored the growth of cyanobacteria harmful algal blooms (CyanoHABs), particularly *Microcystis* spp. (Lehman et al., 2017). Because, the frequency and intensity of drought and associated cyanobacteria blooms are expected to increase with climate change, understanding their impact on fishery production in SFE is critically important (IPCC, 2007).

Microcystis blooms are a major water quality problem in surface waters worldwide. *Microcystis* can produce cyanotoxins, including microcystins, which promote liver cancer in humans and wildlife from freshwater to marine ecosystems (Zegura et al., 2003; International Agency for Research on Cancer, 2006; Ibelings and Havens, 2008; Miller et al., 2010). *Microcystis* also contains lipopolysaccharide endotoxins, which inhibit ion transport in fish gills, as well as fish embryo development (Codd, 2000). *Microcystis* has been associated with negative impacts to the health and survival of fish exposed to microcystins in the diet and zooplankton exposed to both dissolved microcystins in the water and microcystins in the diet (Ger et al., 2009, 2010; Acuña et al., 2012). Various water quality conditions and environmental factors, such as elevated water temperature, high light intensity, and increased nutrients enhanced the growth of *Microcystis* and production of microcystins (Davis et al., 2009; Pineda-Mendoza et al., 2016).

Water quality deterioration due to anthropogenic contaminants can also be enhanced during drought conditions in the SFE. For example, reduced freshwater inflow can enhance pesticide concentrations in surface waters due to concentration effects, because pesticide application in agricultural lands in California has been constant or slightly increasing since 2010 (CADPR, 2017). Herbicides are also sprayed to control invasive aquatic plants, such as water hyacinth (*Eichhornia crassipes*) and *Egeria densa* in the SFE (Santos et al., 2009). Controlling undesirable organisms is economically important, however, the increasing concentrations of anthropogenic chemicals during severe drought years raises environmental concerns on health of endemic aquatic organisms in the SFE, where fishery production has declined since 2000 (Sommer et al., 2007).

Toxicity studies using fish embryos offer a unique perspective on ecological health evaluation. Organisms at the embryonic stage are vulnerable to contaminants because their tissues and vital biological systems are differentiating and developing rapidly. Thus any disruption or impact at the embryonic stage can exert lifelong consequences, such as deformities (McKim, 1977; Embry et al., 2010). The cumulative effect of differentiation and development makes embryos an ideal model for acute and chronic toxicity studies as well (Belanger et al., 2013; Wagner et al., 2017). Fish embryonic toxicity testing has been widely utilized to assess developmental toxicity of various types of chemical compounds, such as cyanotoxins, pesticides, and nanoparticles (Berry et al., 2007; Cho et al., 2013; Alharbi et al., 2016; Wagner et al., 2017).

Despite concerns about the impacts of drought on water quality in the SFE, little is known about how CyanoHAB and contaminants from anthropogenic sources during drought years could impact the health of aquatic species. The State of California Department of Water Resources established a research program to investigate the magnitude, timing, distribution, and food web impact of *Microcystis* in the upper SFE, known as the Sacramento and San Joaquin Delta in the summer of 2014 and 2015, the third and fourth driest years on record in the SFE. As a part of this two-year research program, this study performed fish embryo toxicity testing to determine the potential impact of ambient water quality conditions on the fishery in the SFE. We addressed the hypothesis that contaminants in the water column originating from natural and/or anthropogenic sources during severe drought years lead to deterioration in water quality that impact the health of fishes in the SFE.

2. Materials and methods

2.1. Site description

The SFE contains 1100 km of waterways, which receive freshwater from the Sacramento River on the north, the San Joaquin River on the south, and marine water from the San Francisco Bay on the west (Fig. 1). The water in the estuary is used for agriculture and drinking water and provides habitat for a large suite of aquatic organisms including endangered fish species (Nichols et al., 1986; Brown et al., 2013). *Microcystis* blooms occur in the summer and fall in the SFE, but the length of the bloom season was longer during the drought years (Lehman et al., 2017). *Microcystis* blooms begin in the San Joaquin River and extend both northward and westward into the Sacramento River and Suisun Bay with flow and tide (Lehman et al., 2005). The main river channels are 12 m deep and are linked with shallow water habitats in flooded islands and floodplains that are only a few meters deep.

2.2. Field sampling

Sampling was conducted bi-weekly at 10 stations during the *Microcystis* bloom season in 2014 (between July and December) and 2015 (between August and November; Fig. 1). Water temperature, specific electrical conductance, dissolved oxygen concentration, pH, and turbidity were measured at 0.3 m depth using an YSI 6600 sonde (YSI, https://www.ysi.com/). Specific electrical conductance data were converted to salinity. Water was collected for water quality measurements and fish embryo toxicity testing with a van Dorn sampler at 0.3 m depth (subsurface water samples) and immediately stored on ice. Water for chloride, ammonium, nitrate plus nitrite, silicate and soluble reactive phosphorus measurements was filtered through Nucleopore filters (0.45 μm pore size) and frozen until analysis (United States Environmental Protection Agency, 1983; United States Geological Survey, 1985; American Public Health Association et al., 1998). Water for dissolved organic carbon (DOC) analysis was filtered through precombusted GF/F filters (pore size 0.7 μm) and kept at −20 °C until analysis (American Public Health Association et al., 1998). Unfiltered water samples for total and volatile suspended solids, total organic carbon (TOC), and total phosphate analyses were kept at 4 °C until analysis (American Public Health Association et al., 1998).

Water samples for determination of *Microcystis* biovolume (>75 μm size fraction) and chlorophyll *a* concentration were also collected from the surface of the water column by a gentle hand tow of a 0.3 m diameter plankton net (75 μm mesh) over a distance of 30.5 m. The net was fitted with floats that kept the ring just below the surface, making the net tow an integrated sample of the 0.3 m surface layer. A surface

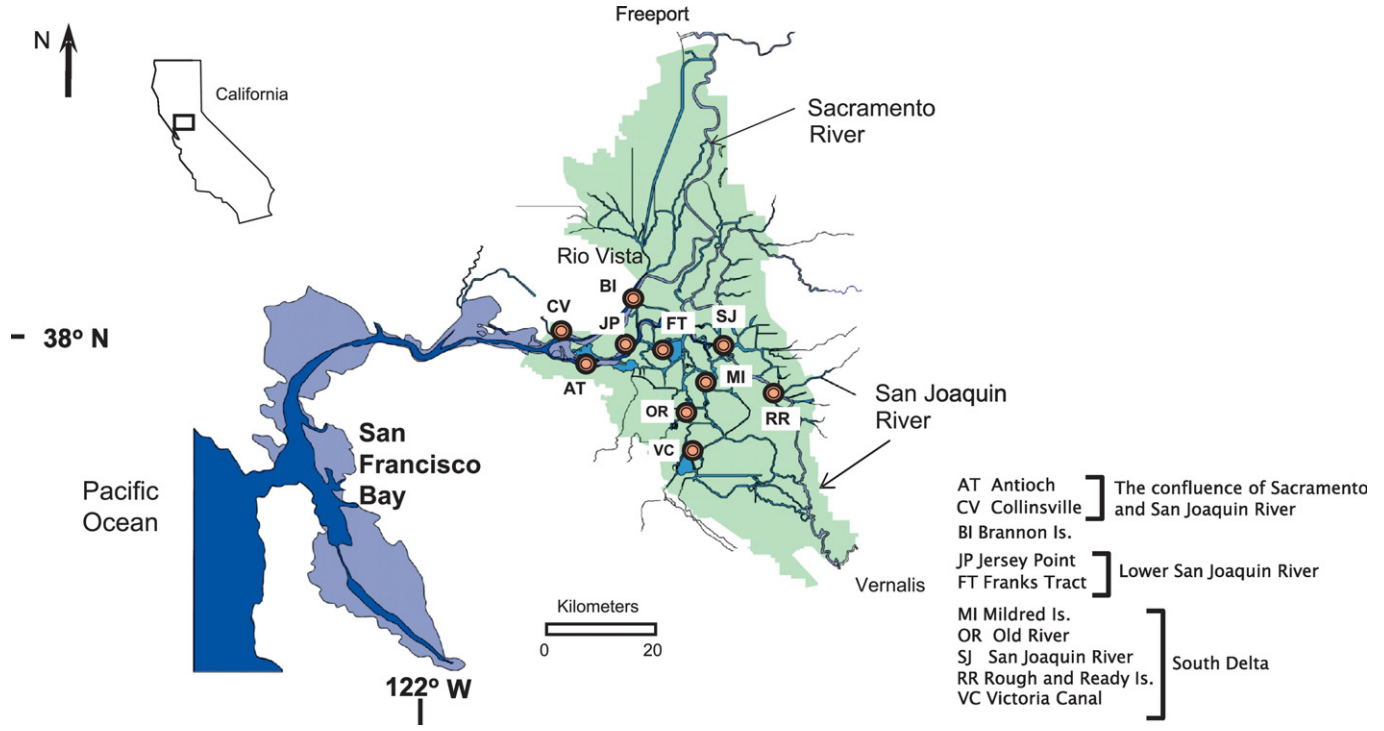

Fig. 1. Map of the San Francisco Estuary showing the location of the sampling stations.

net tow was used in order to get a representative sample of the large *Microcystis* colonies, which were widely dispersed across the surface of the water column and can reach 50,000 μm in diameter. *Microcystis* biovolume and chlorophyll *a* concentration in the net tow were corrected to the total volume of water sampled using a General Oceanics 2030R flow meter.

Samples for determination of *Microcystis* biovolume were preserved with Lugol's solution. The biovolume of *Microcystis* colonies was computed as an area based diameter using a FlowCAM digital imaging flow cytometer (Fluid Imaging Technologies; Sieracki et al., 1998). Cell abundance estimates based on FlowCAM measurements were closely correlated with those determined by microscopic analyses (Lehman et al., 2017). Replicate water samples for chlorophyll *a* and phaeophytin pigment analysis were filtered through GF/F filters (0.7 μm pore size), treated with 1% magnesium carbonate solution to prevent acidity, and frozen until analysis. Pigments were extracted in 90% acetone and quantified using spectrophotometry (American Public Health Association et al., 1998).

Water samples for fish toxicity testing were transported to the Aquatic Health Program at the University of California (UC), Davis. Water samples were filtered twice, first by glass microfiber filter (GF/F, pore size: 1.5 μm, Fisher Scientific, Waltham, MA), followed by an additional filtration with a polyethersulfone syringe filter (PES filter, pore size: 0.22 μm, Genesee Scientific, San Diego, CA) to completely remove particulate organic matter and bacteria. For toxicity testing with the Medaka embryos, exposure to the ambient water was initiated within 48 h of sample collection. For Delta Smelt embryo toxicity testing, filtered water was stored in a −20 °C freezer, for up to five months, until Delta Smelt eggs were available in January.

2.3. Fish embryo toxicity testing

Two fish species were selected for the tests: Delta Smelt (*Hypomesus transpacificus*) and Medaka (*Oryzias latipes*). Delta Smelt is a pelagic fish species endemic to the SFE and is an endangered species that is considered to be an indicator of estuarine health (Moyle et al., 2016). Medaka was used as a fish model for this study because it is a hardy species that is easy to culture, and importantly Medaka is an asynchronous spawner and lays eggs year-round under a controlled laboratory setting while Delta Smelt eggs are available only in spring. Compared to other fish models such as Zebrafish, Medaka embryos have an extended post-fertilization hatch time, which is ideal for investigating developmental effects, because more time is allowed for toxic action to occur (Kinoshita et al., 2009; Wagner et al., 2017).

Fertilized Delta Smelt eggs were provided by Dr. Tien-Chieh Hung at the Fish Conservation and Culture Laboratory at UC Davis. Medaka eggs were collected from in-house mass cultures at the Aquatic Health Program, UC Davis. Fish embryo toxicity testing was performed following a protocol proposed by Braunbeck and Lammer (2006) with some minor modifications. Briefly, fish eggs (<6 h post-fertilization) were collected from aquariums, cleaned in salt solution for 10 min (1%NaCl), incubated in an embryo rearing solution for 30 min (1 g L^{-1} NaCl, 0.030 g L^{-1} KCl, 0.040 g L^{-1} $CaCl_2 \cdot H_2O$, 80 mg L^{-1} $MgSO_4$, and 1 mg L^{-1} methylene blue in distilled water), and then sorted for viable fish eggs. Eggs were placed in 96-well plates filled with twice filtered ambient water (one embryo per well, 200 μL of ambient water per well). A total of 32 eggs were used per water sample per fish species. For control groups, embryos were cultured in the field blank (DI water) with addition of minerals for stabilizing pH and for fish osmoregulation (USEPA, 2002). Fish embryos were kept in an environmental chamber (Percival Scientific, Perry, IA) at optimal growth temperatures for Delta Smelt (16 °C) and Medaka (25 °C) on a 16:8 h light:dark cycle during the exposure period. At 3 days post-exposure, embryos that failed to develop due to poor egg quality were removed from the plates. Approximately 90% of the water in each well was changed every 48 h for 14 days or until hatching. All embryos were observed daily and recorded for signs of abnormal development, mortality, and hatching success. Embryos without visible heartbeats were considered dead. Only Medaka fish embryo toxicity tests were conducted at the peak of the *Microcystis* bloom in August and September of 2014. Both Delta Smelt and Medaka fish embryo toxicity tests were conducted throughout the bloom season in 2015.

2.4. Taxonomic identification of bacteria

Medaka embryos with active bacterial growth (Survey: August 2015; Sampling stations: FT, MI, and SJ; pool of 3 individual embryos per station) were used for identification of bacteria. Medaka embryos with bacterial growth were homogenized in 1 × phosphate buffered saline (PBS). The homogenates were serially diluted with 1 × PBS (1:10 dilutions) and then inoculated onto Luria-Bertani (LB) bacteria culture plates to obtain isolated bacterial colonies. At 2 days post-incubation at room temperature, bacterial colonies on the culture plates were visually examined and the dominant bacterial species accounting for over 90% of the entire bacterial colonies was subjected to taxonomic identification by determining the DNA sequence for the 16S ribosomal RNA gene (16S rDNA), a marker gene that is widely used for bacterial identification (Santamaria et al., 2012; Salman et al., 2013; Boyd et al., 2015). A portion of 16S rDNA, approximately 1500 base pairs, was amplified by a polymerase chain reaction with a generic primer set, EUBA and EUBB with the optimized amplification condition (Gunther et al., 2010). The amplified DNA fragment was extracted from a gel and submitted to the UC DNA Sequencing Facility for DNA sequencing reactions (http://dnaseq.ucdavis.edu/). The DNA sequences were subjected to quality trimming using Geneious software ver. 5.0.4, followed by BLASTN for a DNA sequence similarity search and taxonomic identification (Kearse et al., 2012; http://www.ncbi.nlm.nih.gov/).

2.5. Fish toxicity testing at different salinity levels

An additional fish embryo toxicity test was conducted with Medaka to determine how increased salinity affects mortality. The experimental solutions were prepared by adding Instant Ocean Sea Salt solutions (Instant Ocean, Blacksburg, PA) to ambient water collected from station SJ on August 15th, 2015 to produce salinity of 1.0, 2.5, and 5.0 psu. The dilution of ambient water by salt solution was a concern, therefore the same volume of the Instant Ocean Sea Salt solutions at different concentrations (2 mL) was spiked into the ambient water to standardize the experiment (38 mL of the ambient water, 1:20 dilution). The ambient water from SJ was chosen for the salinity test as it represents water quality in the South Delta where salinity was constantly low (<1.0 psu) and Medaka experienced high mortality (>90%). The ambient water spiked with DI water was used as a control to ensure that high mortality rate was reproducible in this experiment.

2.6. Bacteria growth test with Microcystis lysate

Microcystis cell lysate, a fluid containing contents of lysed *Microcystis* cells, was prepared as follows: *Microcystis* (Strain UTEX LB 2386, non-microcystin producer) obtained from the Culture Collection of Algae at the University of Texas (https://utex.org/pages/about-us) was cultured in CB media (500 mL) with continuous aeration for 10 days in a temperature controlled environmental chamber at 25 °C (Shirai et al., 1989). The algal cells were harvested by centrifugation at 2500 rpm for 10 min at room temperature, followed by 3 rinses with autoclaved DI water and then resuspension in 30 mL of DI water. The algal cells were lysed by a freeze-thaw method; the algal cells were placed in −80 °C for 20 min and then thawed slowly at room temperature. The freeze-thaw cycle was performed 3 times and the algal cells were visually observed under a light microscope. The algal lysate in the dissolved fraction was collected by centrifugation (2500 rpm at room temperature), followed by filtration using PES filters (pore size: 0.22 μm, Genesee Scientific). The DOC concentration was measured by submitting a portion of the algal cell lysate to the Stable Isotope Facility at University of California, Davis (http://stableisotopefacility.ucdavis.edu/). A bacteria growth test was performed by spiking *Aeromonas* into three different concentrations of the *Microcystis* lysate with DOC concentrations of 27.7, 14.7, and 8.5 mg L^{-1}. The *Aeromonas* used for the experiment was isolated from the Medaka toxicity testing. DI water was used as a control. The experiment was performed in triplicate with a total volume of 3 mL in a sterilized plastic tube. The bacterial density was measured by plate culture assay. Briefly, subsamples of culture solutions containing bacteria (50 μL) were collected at the beginning and end of the experiment (0 and 72 h post-inoculation), serially diluted by 1:10 with 1 × PBS, mixed with 60% glycerol (final concentration: 25%), and then stored in −80 °C. The day after collecting the subsamples at 72 h post-inoculation, the subsamples of bacteria culture solutions were slowly defrosted on ice, spread onto LB culture plates, and then the plates were incubated at room temperature until bacterial colonies became visible. The number of bacterial colonies were counted and the results were reported as the number of colony forming units per milliliter of culture solution (cfu mL^{-1}).

2.7. Statistical analysis

Spearman's rank correlation coefficient was used to calculate the correlation between fish embryo mortality and water quality parameters (PRIMR-e software ver. 6; http://www.primer-e.com/). For the bacteria growth tests with different concentrations of *Microcystis* lysate, means and standard error (S.E.) were computed, and the differences among the treatment means were analyzed by one-way ANOVA, followed by post-hoc test by Dunnett's test using a package "multcomp" ver. 1.4-6 in R software ver. 3.3.0 (Hothorn et al., 2010; R Core Team, 2016).

3. Results

3.1. Water quality data

Water quality parameters measured in this study are available in our previous publication (Lehman et al., 2017). In 2014 and 2015, the DOC concentration and water temperatures varied with time and location in the SFE (Fig. 2). Among the 10 sampling stations, the DOC concentration and water temperature were slightly higher at RR than the other locations (Fig. 2). The salinity at the confluence of the rivers was constantly above 1.5 psu (AT and CV; average: 3.7 psu) while the other sampling stations (Lower San Joaquin River and South Delta) had lower salinity levels throughout the surveys (average: 0.6 psu, maximum: 0.8 psu).

3.2. Mortality and adverse effects

In both 2014 and 2015, Medaka embryos incubated in the ambient water from the stations containing *Microcystis* blooms started to exhibit

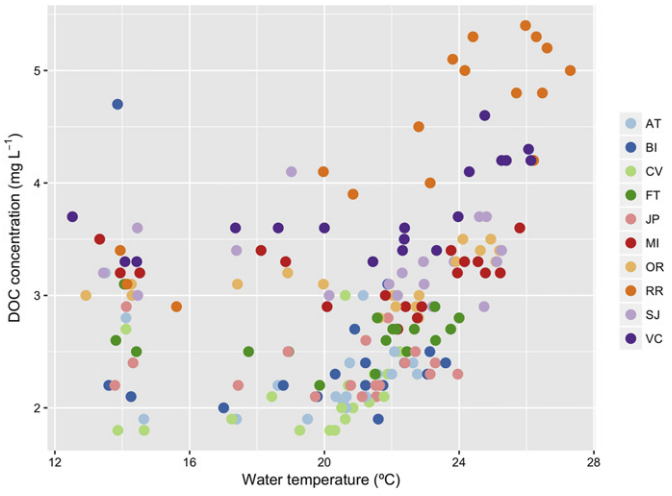

Fig. 2. Scatter plot showing the DOC concentration and water temperature recorded for the 2014 and 2015 surveys.

growth of motile bacteria on the chorion (the outer envelope of the fish egg) of live fish embryos within 5 days of exposure (Fig. 3a). Medaka embryos with bacteria died with a portion of the egg yolk or the entire embryonic body extruded from the chorion (Fig. 3b and c). In 2014, the Medaka mortality rates ranged between 77.4 and 96.9% at the 8 sampling stations located in the Lower San Joaquin River and South Delta (BI, JP, MI, FT, SJ, OR, VC and RR), and below 10% at the confluence of the Sacramento and San Joaquin rivers (AT and CV) for August and September (Table 1a). The high mortality rates of the fish embryos were observed in conjunction with the growth of the bacteria, *Aeromonas*. In 2015, the mortality rates exceeded 90% for all the sampling stations in the Lower San Joaquin River and South Delta in August, and then decreased in the following months (Table 1b). Growth of bacteria was still observed in embryos incubated in October and November samples from the same sampling stations, however not all fish died. In contrast, at the confluence of the rivers (AT and CV), the Medaka mortality rates remained 10% or lower throughout 2015. Both in 2014 and 2015, there was little mortality in the control groups and growth of bacteria was barely observed (<4%; Table 1a,b; Fig. 3e). The Medaka percent mortality was negatively correlated with chloride (Pearson's $r = -0.59$, $P < 0.001$), and was positively correlated with *Microcystis* biovolume ($r = 0.36$, $P < 0.05$), ambient water temperature ($r = 0.58$, $P < 0.001$), DOC ($r = 0.31$, $P < 0.05$), TOC ($r = 0.47$, $P < 0.001$), and surface chlorophyll *a* concentration ($r = 0.34$, $P < 0.05$) (Table 2).

The Delta Smelt mortality rates remained below 20% in the majority of the water samples tested (23/30 or 77%; Table 1c). Similar to Medaka, growth of motile bacteria was associated with Delta Smelt mortality. However, the Delta Smelt mortality rates were lower than that for Medaka, and ranged between 3.1% (multiple stations in September and November 2015) and 41.9% (JP in November 2015; Table 1c). Unlike Medaka, no correlation was observed between Delta Smelt mortality data and ambient water quality parameters (Table 2).

In addition, a developmental deformity "heart-only syndrome" was observed during the toxicity tests for Medaka. The term, "heart-only syndrome", was used to describe the distinctive symptom that occurred when inhibition of somite formation co-occurred with cardiogenesis and beating of a functional heart (Fig. 3d). The frequency of the developmental deformity was low at <0.5% of the fish embryos tests. There was no clear seasonal and geographic trend in the occurrence of the developmental deformity, however, one of the sampling locations in which the developmental deformity observed was located in the middle of large agricultural fields (VC). The deformity was not found in either the control groups or the fish culture facilities.

3.3. Salinity test with Medaka embryos

There were significant reductions in the Medaka mortality rates (≤ 13.3%) at salinity ≥1.0 psu, compared with the same ambient water sample without salt addition (96.7%; Table 3). Growth of the motile bacterial was not observed at salinity ≥2.5 psu. No mortality was observed in the control group.

3.4. Bacteria growth test with Microcystis lysate

The bacterial growth test demonstrated that *Microcystis* lysate enhanced the growth of *Aeromonas* (Fig. 4). The densities of *Aeromonas* increased from $6.3 \times 10^4 \pm 1.4 \times 10^2$ (Mean ± S.E.) cfu mL^{-1} at 0 h post-inoculation (hpi) to $1.46 \times 10^6 \pm 1.25 \times 10^5$, $2.87 \times 10^6 \pm 1.07 \times 10^6$, and $7.40 \times 10^5 \pm 5.44 \times 10^5$ cfu mL^{-1} in the Lysate 1, 2, and 3 treatment at the end of the experiment (72 hpi), respectively. Few bacterial colonies were observed in the control group at 72 hpi. The bacterial densities in Lysate 1 and 2 were statistically significant from the control group.

4. Discussion

The high mortality rates of Medaka embryos were associated with the growth of motile bacteria, *Aeromonas*. *Aeromonas* are ubiquitously present in natural water bodies as well as fish culture facilities, and cause disease, particularly when the abundance of the bacteria is enhanced by abiotic factors (e.g., organic pollutants, water temperature) or the fish immune system is compromised by stressors (e.g., inadequate water quality, low dissolved oxygen) (Janda and Abbott, 2010; White, 2013). Mass mortality events caused by *Aeromonas* have been widely reported in the wild, as well as cultured fish populations (Harikrishnan and Balasundaram, 2005). The adverse effects observed in Medaka embryos, such as extrusion of the egg yolk and fish body from the chorion, were likely due to *Aeromonas* since a thick bacterial layer was observed on the affected fish embryos. Adult fish infected with *Aeromonas* exhibited skin ulcers, indicating that *Aeromonas* erodes fish tissue (Harikrishnan and Balasundaram, 2005). Although speculative, given the effects of *Aeromonas* on the epidermal tissue of adult fish, it is possible that the adverse effects observed in this study were due to *Aeromonas* eroding the chorion layer of the fish embryos.

It is suspected that *Aeromonas* originated from the fish culture facility and were introduced into the study from the embryos, because the ambient water samples were filtered twice (1.7 and 0.22 μm) prior to the exposure tests to completely remove any bacteria. Although Medaka eggs were treated with salt solution to lower bacterial loads prior to the fish embryo toxicity testing, the cleaning process may be insufficient in completely removing bacteria on fish eggs. However, because Medaka embryos in control groups showed little to no mortality, we concluded that the growth of *Aeromonas* was enhanced by dissolved substances in the ambient water.

A plausible explanation for the growth of *Aeromonas* in Medaka tests is the presence of dissolved substances, particularly the organic carbon released from *Microcystis* cells (e.g. cell organelle, lipopolysaccharide, or cyanotoxins). The Medaka mortality data showed a positive correlation with *Microcystis* biovolume, TOC or DOC concentration, and other variables that are related to *Microcystis* blooms, such as ambient and net chlorophyll *a* concentration, and water temperature. We also found that the growth of *Aeromonas* was significantly enhanced by dissolved organic substances extracted from *Microcystis* cells. Dissolved organic carbon is often the primary substrate for bacterial growth in

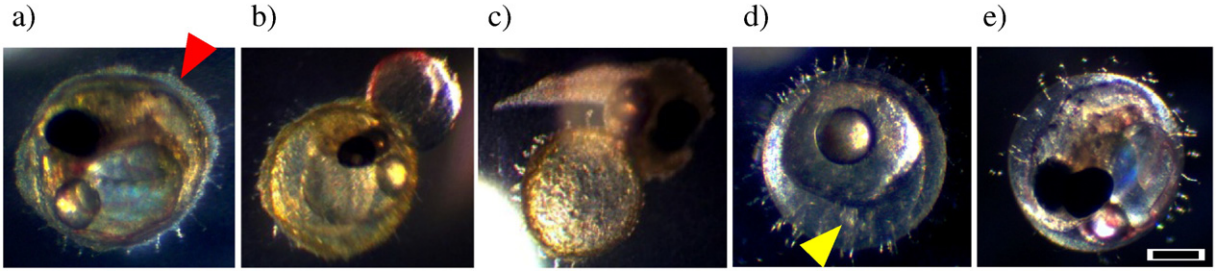

Fig. 3. Images of Medaka embryo incubated in the ambient water samples showing growth of bacteria on the chorion as indicated by the red arrow head (a), extruded egg yolk (b) and whole embryonic body from the chorion (c), inhibition of somite formation while a functional heart was observed as indicated by the yellow arrow head (d), and normal Medaka embryo in control group at 8 days post-fertilization (e). Scale bar: 3 mm.

Table 1
Summary of the fish toxicity testing results for Medaka 2014 (a) and 2015 (b) and Delta Smelt 2015 (c).

(a)

Treatment No.	Station	August			September		
		Mort[a]	Total[b]	Mort (%)[c]	Mort	Total	Mort (%)
1	AT	3	31	9.7	2	32	6.3
2	CV	1	32	3.1	1	31	3.2
3	BI	28	32	87.5	30	31	96.8
4	FT	27	32	84.4	28	32	87.5
5	JP	31	32	96.9	24	31	77.4
6	OR	30	32	93.8	30	31	96.8
7	MI	27	31	87.1	30	31	96.8
8	SJ	30	32	93.8	27	32	84.4
9	RR	28	31	90.3	25	32	78.1
10	VC	29	32	90.6	28	32	87.5
Control	Field control	0	32	0	1	32	3.1

(b)

Treatment No.	Station	August			September			October			November		
		Mort	Total	Mort (%)	Mort	Total	Mort (%)	Mort	Total	Mort (%)	Mort	Total	Mort (%)
1	AT	3	30	10.0	2	31	6.5	1	32	3.1	1	32	3.1
2	CV	2	32	6.3	1	31	3.2	1	32	3.1	0	32	0
3	BI	31	31	100	2	32	6.3	3	32	9.4	5	31	16.1
4	FT	32	32	100	7	32	21.9	9	32	28.1	5	31	16.1
5	JP	32	32	100	4	31	12.9	3	32	9.4	6	29	20.7
6	OR	32	32	100	3	31	9.7	14	32	43.8	1	27	3.7
7	MI	29	30	96.7	9	31	29.0	10	32	31.3	9	30	30.0
8	SJ	28	28	100	5	31	16.1	14	32	43.8	5	32	15.6
9	RR	27	29	93.1	2	32	6.3	19	32	59.4	9	32	28.1
10	VC	31	31	100	6	32	18.8	11	32	34.4	3	29	10.3
Replicate	AT	2	32	6.3	1	31	3.2	3	32	9.4	0	30	0
Control	Field control	1	31	3.2	0	30	0	1	32	3.1	1	30	3.3

(c)

Treatment No.	Station	September			October			November		
		Mort [a]	Total [b]	Mort (%)	Mort	Total	Mort (%)	Mort	Total	Mort (%)
1	AT	4	32	12.5	4	32	12.5	3	32	9.4
2	CV	3	32	9.4	6	31	19.4	2	32	6.3
3	BI	3	32	9.4	8	32	25.0	1	32	3.1
4	FT	7	31	22.6	12	32	37.5	9	32	28.1
5	JP	4	32	12.5	2	32	6.3	13	31	41.9
6	OR	1	32	3.1	5	32	15.6	1	32	3.1
7	MI	6	32	18.8	6	32	18.8	5	32	15.6
8	SJ	6	32	18.8	7	32	21.9	5	32	15.6
9	RR	11	32	34.4	6	32	18.8	6	32	18.8
10	VC	1	32	3.1	6	32	18.8	4	32	12.5
Replicate	AT	3	32	9.4	4	32	12.5	5	32	15.6
Control	Field control	3	32	9.4	6	32	18.8	5	32	15.6

[a] Number of mortalities.
[b] Total number of embryos.
[c] Percentage of mortality.

aquatic ecosystems (Williamson et al., 1999). Furthermore, DOC released from phytoplankton and cyanobacteria is considered to be high quality carbon for bacterial growth (Eiler et al., 2003; Bade et al., 2007). Guillemette et al. (2016) also reported that bacterial communities prefer to utilize algal carbon even in lakes that are dominated by terrestrial carbon.

Both allochthonous inputs of terrestrial materials and autochthonous inputs by phytoplankton, benthic algae, and aquatic macrophytes all contribute to the TOC and DOC in aquatic ecosystems (Volk et al., 2002; Aitkenhead-Peterson et al., 2003; Bertilsson and Jones, 2003; Bade et al., 2007). However, the DOC in the SFE mainly originated from phytoplankton or cyanobacteria and not terrestrial organic matter as demonstrated by stable isotopic analysis (Lehman et al., 2015). In 2014, the largest biomass of *Microcystis* was observed since the blooms began in 1999, with median chlorophyll *a* concentration reaching levels that were 13 and 9 times greater than in previous wet and dry years, respectively (Lehman et al., 2017). In addition, dissolved organic substances could have been released from other cyanobacteria. In 2014, dissolved organic carbon concentration was significantly correlated with the abundance of the cyanobacterium, *Aphanizomenon* (Spearman's $r = 0.33$; $P < 0.01$, Lehman et al., 2017). All these results suggest that blooms of *Microcystis* and other cyanobacteria species can contribute to the concentration of organic carbon in ambient waters, and therefore, will increase the potential growth of bacterial as CyanoHABs become more intense.

Difference in water temperature may potentially explain the high mortality rates for Medaka compared with the low mortality rates for Delta Smelt. In the SFE, spawning of wild Delta Smelt occurs in spring when the water temperature is between 7 and 15 °C (Bennett, 2005). During the exposure tests, Delta Smelt embryos were maintained at the optimum temperature of 16 °C for their normal development. This water temperature was significantly lower than the optimum for the growth of *Aeromonas* at 28 °C (Statner et al., 1988). Rouf and Rigney (1971) reported that the growth of *Aeromonas* significantly decreased at 15 °C. In contrast, Medaka tests were performed at 25 °C, which is close to the optimum for *Aeromonas* growth. Although *Aeromonas*

Table 2
Spearman correlation coefficients computed between fish mortality data and water quality parameters.

		20	19	18	17	16	15	14	13	12	11	10	9	8	7	6	5	4	3	2
		MIC	Phae, sub	Chl-*a*	Chl-*a*, sub	TDS	TSS	DON	DOC	TOC	TP	SRP	NO3	NH4	pH	Turb	CL	DO	WT	MK
1	Delta Smelt mortality	(DS)	0.10	−0.02	0.04	0.17	−0.25	−0.16	0.20	0.23	0.21	0.32	0.31	0.30	0.25	0.01	−0.04	−0.26	0.16	0.11	0.33
2	Medaka mortality	(MK)	**0.36***	0.06	**0.34***	0.21	**−0.58****	**−0.55****	−0.12	**0.31***	**0.47****	0.09	0.16	−0.09	**−0.29***	**0.39***	**−0.35***	**−0.59****	**−0.27***	**0.58****	
3	Water temperature	(WT)	**0.28***	0.21	−0.02	−0.05	**−0.26***	**−0.29***	0.05	**0.35****	**0.45****	**0.26***	**0.29***	0.03	**−0.27***	0.12	−0.14	**−0.27***	**−0.57****		
4	Dissolved oxygen	(DO)	**−0.36***	−0.22	0.07	0.11	0.04	0.01	−0.07	−0.15	**−0.24***	−0.09	−0.12	0.06	**0.30***	**0.39****	−0.07	0.03			
5	Chloride	(CL)	−0.10	0.03	**−0.35***	**−0.51****	**0.99****	**0.79****	0.08	**−0.48****	**−0.56****	−0.07	−0.11	0.01	0.17	**−0.36***	**0.66****				
6	Turbidity	(Turb)	−0.02	0.17	−0.21	**−0.40***	**0.66****	**0.70****	−0.03	**−0.41****	**−0.45****	−0.08	−0.10	−0.02	0.13	**−0.45****					
7	pH	(pH)	0.13	−0.19	**0.28***	0.09	**−0.36***	**−0.30***	−0.03	0.08	0.05	−0.13	**−0.60****	−0.10	−0.19						
8	Ammonium	(NH4)	−0.21	**0.39***	−0.18	−0.10	0.17	**0.24***	**0.25***	**0.26***	0.22	**0.67****	**0.60****	**0.77****							
9	Nitrate	(NO3)	−0.18	**0.48****	−0.11	0.08	0.02	0.04	**0.36***	**0.54****	**0.52****	**0.94****	**0.93****								
10	Soluble reactive phosphorus	(SRP)	−0.05	**0.59****	0.00	0.11	−0.10	−0.12	**0.33****	0.67	**0.68****	**0.97****									
11	Total phosphorus	(TP)	−0.07	**0.62****	−0.05	0.11	−0.06	−0.08	**0.36***	**0.67****	**0.67****										
12	Total organic carbon	(TOC)	0.21	**0.44****	**0.23***	**0.50****	**−0.56****	**−0.59****	**0.27***	**0.83****											
13	Dissolved organic carbon	(DOC)	0.19	**0.32***	0.18	**0.32***	**−0.48****	**−0.51****	**0.27***												
14	Dissolved organic nitrogen	(DON)	**0.29***	**0.36***	0.09	**0.28***	0.08	0.12													
15	Total suspended solids	(TSS)	0.02	0.24	**−0.28***	**−0.48****	**0.79****														
16	Total dissolved solids	(TDS)	−0.11	0.04	**−0.35***	**−0.51****															
17	Sub-surface chlorophyll *a*	(Chl-*a*, sub)	0.22	0.20	**0.68****																
18	Surface chlorophyll *a*	(Chl-*a*)	**0.42****	0.05																	
19	Sub-surface phaeophytin	(Phae, sub)	0.09																		
20	Microcystis biovolume	(MIC)																			

The numbers in bold indicate $P < 0.05$.
* $P < 0.05$.
** $P < 0.001$.

Table 3
Mortality of Medaka embryos cultured in ambient water at different salinity levels.

Treatment No.	Station	Salinity (psu)	Mort[a]	Total[b]	Mort (%)[c]
1	SJ[d]	<0.1	29	30	96.7
2	SJ	1.0	4	30	13.3
3	SJ	2.5	0	31	0
4	SJ	5.0	0	32	0
Control	Field control	<0.1	0	31	0

[a] Number of mortalities.
[b] Total number of embryos.
[c] Percentage of mortality.
[d] Ambient water collected at SJ in August 2015.

growth at different temperatures was not tested in this study, the difference in the water temperature used for the two fish species likely affected the mortality results.

We consider the high Medaka mortality due to bacterial growth to be a potentially important factor affecting the impact of future drought conditions and associated *Microcystis* blooms on fish species in the SFE, particularly in the freshwater regions. Spawning of American Shad (*Alosa sapidissima*) and Threadfin Shad (*Dorosoma petenense*) occur between late April and August in the San Joaquin River and, produce large numbers of young fish that remain in the freshwater regions of the estuary until November (Walburg and Nichols, 1967; Feyrer et al., 2009). During these months, embryos and early life stage fish could be impacted by pathogenic bacteria such as *Aeromonas*. Among the 10 sampling stations monitored in this study, the DOC concentrations and water temperatures at RR were relatively higher than those in the other stations (Fig. 2). The warmer water temperatures (>25 °C) and the DOC concentrations (>4.5 mg L^{-1}) might have enhanced growth of *Aeromonas* and any other bacterial species during the severe drought years. Currently there is no official data reporting biodiversity and abundance of bacteria in surface water in the SFE. *Aeromonas* was found to be the dominant species in fish embryo toxicity testing, however, this result may not represent bacterial assemblages in the fields. Further research is strongly warranted to investigate the contribution of the DOC originating from CyanoHABs on the growth of pathogenic bacteria in the field.

The finding of a developmental deformity observed in Medaka embryos, "heart-only syndrome", also supports the importance of investigating natural and anthropogenic contaminants in the ambient water. Developmental toxicities associated with cyanotoxins from *Oscillatoria*, *Plectonema*, *Calothrix*, and *Fischerella* include the arrest of embryonic development at mid-blastula stage, development of pericardial peritoneal

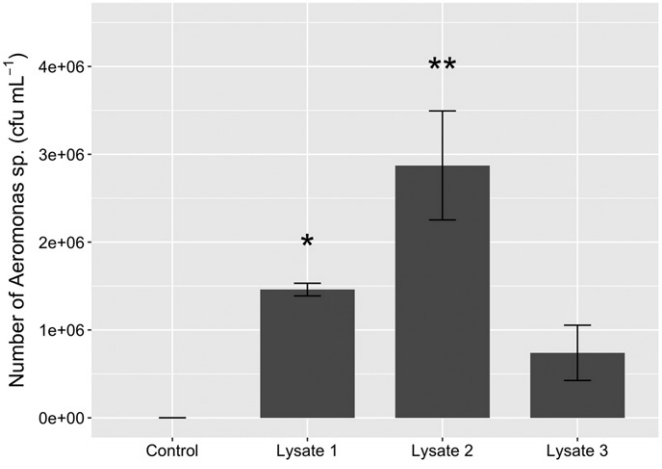

Fig. 4. Bar plot showing abundance of *Aeromonas* sp. (colony forming unit mL^{-1} of culture solution) cultured with different concentrations of *Microcystis* lysate: Lysate 1 (DOC concentration: 27.7 mg L^{-1}), 2 (14.7 mg L^{-1}), and 3 (8.5 mg L^{-1}). The bacterial abundance was measured at 72 h post-inoculation. (*$P < 0.05$, **$P < 0.001$).

edemas, and inhibition of organogenesis (Berry et al., 2007). Similarly, anthropogenic contaminants also caused morphological abnormalities in fish embryos. Pamanji et al. (2015) reported that Zebrafish exposed to profenofos, an organophosphate insecticide, exhibited various morphological deformities including yolk sac edema and malformations in the tail and head. The cause of the developmental deformity, "heart-only syndrome" is still unknown, however, association of natural or anthropogenic contaminants is suspected. Further investigation is warranted to identify the cause of the deformity and assess risk of contaminants in the aquatic environment.

5. Conclusion

In this study we found that dissolved substances released from *Microcystis* promoted the growth of *Aeromonas*, which resulted in high mortality rates in Medaka embryos. The increased frequency of *Microcystis* and other CyanoHAB species are a global concern due to the impact of cyanotoxins, however, our findings suggest that intense algal blooms may also be another cause of water quality deterioration by enhancing bacterial growth in aquatic environments. Elevation of water temperature and dissolved organic matter from intense CyanoHABs during drought can provide favorable condition for bacteria, which can negatively affect indigenous fish species in the aquatic ecosystem. All the experiments were carried out under a controlled laboratory setting, therefore our knowledge on impacts of drought on the aquatic environment is still limited. Particularly, dissolved substances released from *Microcystis* may promote other bacterial species in the field. Further investigation is warranted to investigate abundance and biodiversity of bacteria in the SFE, and to assess effects of bacteria on Delta Smelt or other native fish species under environmentally relevant condition. Furthermore, contaminants that are responsible for the adverse effects, "heart-only syndrome" observed in this study need to be identified for protection of ecological and environmental health.

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